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TWO METHODS FOR OBTAINING A VIRUS OF RABIES, FREED FROM THE CELLS OF THE HOST AND FROM CONTAMINATING ORGANISMS, AND THE APPLICATION OF THESE METHODS TO OTHER FILTERABLE VIRUSES OR GLYCERIN-EXTRACTS.*†

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We have used two methods for obtaining a virus of rabies separated from the tissue cells of the host and freed from contaminating organisms. These methods we believe to be of value for other viruses, as for example, those of vaccinia, variola, epidemic-poliomyelitis, etc., and also of value in certain glycerin preparations or extracts such as tuberculin.

The first method is by glycerin-extraction, the second by aspiration.

GLYCERIN-EXTRACTION.

Glycerin has long been used for destroying contaminating organisms and preserving the virus of rabies and vaccinia, etc., and of epidemic-poliomyelitis, more recently by Landsteiner, Levaditi, and Pastia.[1]

Glycerin is also of great value in certain extracts of bacteria and other preparations as in tuberculin. With tuberculin, several methods have been used for ridding it of glycerin, and thus allowing of a dry tuberculin: one is precipitation by alcohol and ether, which is costly; another is dialysis through parchment membrane. This dialysis is a comparatively slow process, difficult to perform under sterile conditions, and considerable of the tuberculin is lost.

No method, so far as we know, has been adopted for removing the glycerin from the viruses, after it has accomplished its

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* Received for publication February 10, 1913.

† Read at the meeting of the American Association of Pathologists and Bacteriologists in Philadelphia, April, 1912.

[1] *Ann. de l' Inst. Pasteur,* 1911, 25, p. 805.

purpose of preservation or destruction of contaminating organisms. Certain experiments on the nature of these viruses are complicated or rendered impossible by the presence of glycerin.

One of us[1] found that the virus of rabies could be extracted with glycerin from the submaxillary glands of rabid dogs. The glands were taken from the head under aseptic precautions. After removal of the fibrous sheath, they were immersed in sterile neutral glycerin and allowed to stand in the icebox for 6 to 12 days. The clear supernatant glycerin, slightly colored by hemoglobin, was then pipetted off. This was usually found to be sterile, and microscopical examination of this fluid showed practically nothing demonstrable in the way of formed cellular elements. This glycerin-extract retained its virulence, when kept in the icebox, for a very long time, in one experiment 191 days.

No further work was done on this extract, due to the limitation of experimentation with a glycerin virus.

By our present method—dialysis through collodion sacs—we are able to get rid of the glycerin and have our virus uncontaminated in any fluid we select.

We do not say that the dialysis of glycerin is a new method, as it is a well known fact, but its application, as far as we can ascertain, has been through parchment or parchment paper. The objections to this we have already mentioned in connection with tuberculin.

By the use of the collodion sacs, all the operations are carried on under aseptic conditions. The usual contaminating organisms do not pass through a collodion sac. Therefore if the sac is sterile and the glycerin extract put in the sac is pure, the fluid in which it is placed for dialysis need not be sterile.

Another advantage of the collodion sacs is that dialysis of glycerin takes place very rapidly in physiological salt solution, Ringer's solution, or distilled water. For example, the glycerin, from 4–5 c.c. of the glycerin virus of rabies, could be removed in one to two hours' dialysis in running salt solution.

A third advantage of the collodion sac is that it may also be used to concentrate the virus. After dialysis the virus is diluted,

[1] Poor, *Proc. of the New York Path. Soc.*, 1906, 6, p. 85.

the dilution varying with the amount of water the glycerin has previously extracted from the tissues.

In microscopical studies or other work, a concentration may be desired. Evaporation may not be practicable, and as most of the filterable viruses do not filter through a collodion sac, by using the sac as a filter we are able to get rid of the diluting fluid. Although some of the virus may adhere to the side of the sac, a certain concentration must result.

It is true, also, that, although the protein in solution in the sac will gradually pass through as the filtration continues, due to the "coating" of the sac, the protein content of the fluid, remaining within the sac, when the concentration has reached the desired degree, will be increased, as shown by one of us.[1] In interpreting some of the results of concentration, this fact must be borne in mind.

The technic used for making the sacs was that described by Novy[2] and modified by Gorsline.[3]

The sacs were made over a tube which had a small opening in the bottom. This tube was turned several times in a dilute collodion. This collodion was then stripped off by forcing water through the tube. A glass tube was inserted into the neck of the sac, and the junction strengthened by a ligature or a coating of collodion. The sac was filled with distilled water, autoclaved at 105 degrees for 15 minutes, allowed to cool, then emptied, immersed in sterile water, and subjected to pressure (about three inches of mercury). If there was no evidence of leakage, the sac was used. The sacs were without flaws or air bubbles. For dialysis, they were fairly thick; for filtration, they were very thin.

THE ASPIRATION METHOD.

This method was also used by us in our studies on the rabies virus of the submaxillary glands. The glands, after being cut, were placed in distilled water and subjected to a vacuum of 29 inches of Hg for an hour. The pieces of the glands were then pressed in an ordinary meat press and the expressed fluid centrifuged.

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[1] Steinhardt, *Jour. Infect. Dis.*, 1910, 7, p. 675.

[2] *Bact. Lab. Handbook*, 1898.

[3] *Contrib. Med. Res.*, Ann Arbor, Mich., 1903, p. 390.

The supernatant fluid was pipetted off and added to the water in which the glands were aspirated. It may be noted that the aspiration fluid alone was found to contain the virus, but more was present on the addition of the pressed-out juice. By this method a suspension of the virus was obtained in distilled water, which was stronger than the glycerin virus, but which was not sterile and which contained fragments of glands, cells, blood-tissue elements, etc. These were removed by passing it through a Berkefeld filter, the filtrate containing the virus, freed from tissue cells and contaminating bacteria.

CONCLUSIONS.

Our two new methods are: first, the application of collodion sacs for the dialysis of glycerin; second, the use of aspiration for obtaining the virus of rabies from the submaxillary glands of rabid dogs. This aspiration extract is virulent when filtered through a Berkefeld filter.

These methods have enabled us to obtain a virus of rabies freed from the cells of the host and other contaminating organisms and to suspend this virus in any desired fluid.

The results of our studies on rabies will be published shortly. We have also done some comparative work with vaccinia, and are beginning similar studies with epidemic-poliomyelitis, the virus of which, as frequently noted, has a strong likeness to that of rabies.

For obtaining a dry tuberculin from the glycerin preparation, we believe the use of the collodion sacs for dialysis to be superior to other methods.